

Mouse 2895-2898

McAb to NOS3

human iNOS CT: CR(Nk)(Om)-SLEMSAL/Thyro
started 4/20/93

① 1st immunization IP \pm FCA

Note: 0.4 ml aqueous } microtuge
+ 0.4 ml FCA } tube

0.8 ml emulsion, then 0.2 ml/mouse

② 2nd immunization IP \pm FCA

5-12-93

③ 3rd IP \pm FCA

6-2-93

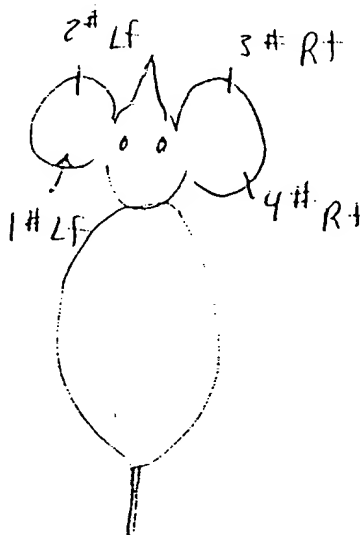
④ numbering order

1st - Lf bottom

2nd - Lf top

3rd - Rt top

4th - Rt bottom



⑤ Test bleed from eye orbital vein 6-9-93

⑥ 4th IP \pm FCA

JC/AF

6-22-93

⑦ 5th IP \pm FCA

JC/RW

10-19-93

⑧ Test bleed from eye orbital vein

AF

10-27-93

⑨ Mice did well with many bleed tests, blood clot speed test

Agarose gel electrophoresis, immunoblotting, RW

⑩ Mice did well with many bleed tests, blood clot speed test

11-21-93

EASY BEAM

Plate # 1 N053 Mico 6 / 1B / 93 Date 5 Filter nm
Operator Comment

	1	2	3	4	5	6	7	8	9	10	11	12	
M 2895 A	+0.008	+1.449	+0.948	+0.589	+0.353	+0.201	+0.101	+0.042	+0.032	+0.022	+0.022	+0.009	A 1g
M 2895 B	+0.031	+0.563	+0.343	+0.212	+0.150	+0.097	+0.060	+0.038	+0.027	+0.023	+0.020	+0.022	B 1gM
M 2896 C	+0.000	+0.010	+0.000	+0.000	-0.005	-0.008	-0.012	-0.008	-0.018	-0.012	-0.007	+0.000	C 1g
M 2896 D	+0.021	+0.084	+0.051	+0.038	+0.023	+0.023	+0.013	+0.013	+0.010	+0.010	+0.008	+0.015	D 1gM
M 2897 E	+0.000	+0.012	+0.000	+0.000	-0.006	-0.008	-0.008	-0.006	-0.010	-0.013	-0.011	+0.000	E 1g
M 2897 F	+0.024	+0.255	+0.136	+0.089	+0.051	+0.035	+0.026	+0.026	+0.017	+0.013	+0.011	+0.011	F 1gM
M 2898 G	+0.011	+0.213	+0.107	+0.064	+0.035	+0.017	+0.000	+0.000	+0.000	-0.006	-0.010	-0.010	G 1g
M 2898 H	+0.028	+0.250	+0.148	+0.109	+0.067	+0.048	+0.036	+0.031	+0.021	+0.021	+0.016	+0.022	H 1gM
blank		100	200	400	800	1600	3200	6400	12800	25600	51200	102400	

M2896 - KK
- KK

EASY BEAM

Plate # 1 1053 Mice Date 6 / 18 / 93 Filter _____ nm
Operator _____ Comment _____

		1	2	3	4	5	6	7	8	9	10	11	12	
M 2895	A	+0.008	+1.449	+0.948	+0.589	+0.353	+0.201	+0.101	+0.042	+0.032	+0.022	+0.022	+0.009	A IgG
M 2895	B	+0.031	+0.563	+0.343	+0.212	+0.150	+0.097	+0.060	+0.038	+0.027	+0.023	+0.020	+0.022	B IgM
M 2896	C	+0.000	+0.010	+0.000	+0.000	-0.005	-0.008	-0.012	-0.008	-0.018	-0.012	-0.007	+0.000	C IgG
M 2896	D	+0.021	+0.084	+0.051	+0.038	+0.023	+0.023	+0.013	+0.013	+0.010	+0.010	+0.008	+0.015	D IgM
M 2897	E	+0.000	+0.012	+0.000	+0.000	-0.006	-0.008	-0.008	-0.006	-0.010	-0.013	-0.011	+0.000	E IgG
M 2897	F	+0.024	+0.255	+0.136	+0.089	+0.051	+0.035	+0.026	+0.026	+0.017	+0.013	+0.011	+0.011	F IgM
M 2898	G	+0.011	+0.213	+0.107	+0.064	+0.035	+0.017	+0.000	+0.000	+0.000	-0.006	-0.010	-0.010	G IgG
M 2898	H	+0.028	+0.230	+0.148	+0.109	+0.067	+0.048	+0.036	+0.031	+0.021	+0.021	+0.016	+0.022	H IgM
		1	2	3	4	5	6	7	8	9	10	11	12	
		blank	100	200	400	800	1600	3200	6400	12800	25600	51200	102400	

M2896 - ~~Q~~KK
M2897 - KK

EASY BEAM

Plate # N053 - mice Date 10 / 28 / 93 Filter _____ nm
 Operator _____ Comment N053 Mice - Test Bleeds

	1	2	3	4	5	6	7	8	9	10	11	12	
M2895 A	+0.013	<u>OVER</u>	+1.913	+1.740	+1.300	+0.783	+0.442	+0.217	+0.127	+0.063	+0.032	+0.014	A
M2896 B	+0.009	+0.133	+0.051	+0.049	+0.030	+0.026	+0.020	+0.018	+0.018	+0.008	+0.008	+0.005	B
M2897 C	+0.000	+0.308	+0.168	+0.106	+0.064	+0.047	+0.029	+0.034	+0.023	+0.006	+0.010	+0.000	C
M2898 D	+0.008	+0.397	+0.270	+0.165	+0.111	+0.068	+0.047	+0.034	+0.020	+0.020	+0.013	+0.010	D
E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
	1	2	3	4	5	6	7	8	9	10	11	12	
	Blank	1:100	1:200	1:400	1:800	1:1600	1:3200	1:6400	1:12800	1:25600	1:51200	1:102400	

y3.1 3XT-75: p8 passage

① Counted cells - 15.5 ml @ 1:2 dilution

Total cells = 49

$$\frac{\text{dead}}{\text{viable}} = \frac{1}{48} \quad (49 \times 10^4)(2)(15.5 \text{ ml}) = 15.19 \times 10^6 \text{ cells per flask}$$

@ 97.9% viability

 $8.4 \times 10^6 \rightarrow 15.9 \times 10^6$ cells in ~ 24 hours \rightarrow 1 doubling cycle in ~ 24 hours

Box 5E

 ② Freeze down cells - previously grown up from a Box 5B vial
 $(15.19 \times 10^6 \text{ cells})(3) = 45.57 \times 10^6 \text{ cells @ } \sim 97\% \text{ viability}$

 ↳ assume cell count is same for all
 3 flasks from 11/24/93 split

 ③ Freeze down 13 vials + 1 test vial (will thaw test vial after cells have been in liq N_2 for 21 days to check viability)
④ 3×10^6 cells frozen per vial

PC-12

① Made new media using heat inactivated horse seraRPMI 1640, 10% Δ H.I. horse sera, 5% FCS 2/1/93② Split pl 1 \rightarrow 3 for propagation

Dislodged cells using a streamer needle from pipet

Wednesday, 1 December 1993

N053 FUSION

SP2/0 4g:4

① Flask #3:

Total cell = 49

- dead = 3

46 viable

 $\rightarrow [4.31 \times 10^6 \text{ cells @ } 99\% \text{ viability}]$

Flock #1

Total cell count = 36

- dead = 2

viable = 34

 3.17×10^7 cells @ 94.4% viability

Flock #1 Total cell count = 40

- dead = 2

viable = 38

 3.52×10^7 cells @ 95% viability

SP240 - Total = $3.52 \times 10^7 + 3.17 \times 10^7 + 4.31 \times 10^7 = 1.1 \times 10^8$

Reseeded 12 X175 with 1×10^7 cells, distributed among 12 flasks

N053

(mouse) Fusion (in vitro imm. spleno's)

FUSION:

① Fused 1×10^6 SP240 with splenocytes in T-75 that were immunized in vitro. Assume $\sim 2 \times 10^6$ splenocytes. Fusion ratio of myeloma to splenocyte = 2:1

② Plated out 5 x 96 well plates

EASY BEAM

Plate # 1 Date 12 / 23 / 93 Filter 492 nm
 Operator DT Comment N053(M)

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	A
B	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	C
D	+0.000	-0.005	-0.007	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	D
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	E
F	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	G
H	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # 2
Operator DT

Date 12 / 23 / 93
Comment N053(M)

Filter 492 nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.000	+0.000	+0.028	+0.014	+0.055	-0.006	+0.000	+0.000	+0.013	+0.000	+0.000	A
B	+0.000	+0.000	-0.007	-0.007	+0.036	<u>+0.337</u>	+0.007	+0.064	+0.000	+0.000	+0.000	+0.000	B
C	-0.005	+0.000	-0.009	-0.007	+0.000	+0.021	+0.000	+0.000	-0.005	+0.000	-0.006	+0.000	C
D	-0.006	-0.008	-0.008	-0.006	+0.000	+0.041	+0.015	+0.000	-0.005	+0.000	+0.000	+0.000	D
E	+0.000	+0.000	+0.000	-0.006	-0.006	-0.010	-0.008	-0.006	-0.008	-0.006	-0.006	+0.000	E
F	+0.000	-0.006	<u><u>7</u></u> <u>+0.040</u>	-0.006	+0.000	-0.006	-0.006	+0.000	+0.000	-0.018	-0.006	+0.000	F
G	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.000	+0.010	+0.014	+0.005	+0.011	+0.008	+0.000	+0.012	+0.006	+0.006	H
	1	2	3	4	5	6	7	8	9	10	11	12	

2 F3 looks darker than 0.040 reading

overlight very strong 2 F3

EASY BEAM

Plate # 3 Date 12 / 23 / 93 Filter 492 nm
 Operator DT Comment N053 (M)

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.019	+0.000	+0.000	A
B	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.007	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.005	D
E	+0.005	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	E
F	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	G
H	+0.015	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.000	+0.008	+0.005	+0.008	+0.010	H
	1	2	3	4	5	6	7	8	9	10	11	12	

EASY BEAM

Plate # 4
Operator DT

Date 12 / 23 / 93
Comment N053(M)

Filter 492 nm

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.006	+0.000	+0.000	+0.000	+0.000	+0.000	+0.013	+0.000	+0.000	+0.000	+0.000	+0.000	A
B	+0.000	+0.000	+0.000	+0.006	+0.000	+0.000	-0.008	+0.000	+0.000	+0.000	+0.006	+0.000	B
C	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.005	D
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	E
F	+0.000	+0.000	+0.000	<u>+0.066</u>	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.005	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.005	+0.005	G
H	+0.025	+0.010	+0.000	+0.000	+0.006	+0.005	+0.008	+0.000	+0.005	+0.000	+0.025	+0.006	H
	1	2	3	4	5	6	7	8	9	10	11	12	

overwight very strong YFY

EASY BEAM

Plate # 5 Date 12 / 23 / 93 Filter 492 nm
 Operator DT Comment N053(M)

	1	2	3	4	5	6	7	8	9	10	11	12	
A	+0.000	+0.008	+0.000	+0.013	-0.007	-0.005	+0.000	+0.000	+0.000	+0.000	-0.006	+0.000	A
B	+0.009	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.008	+0.000	+0.005	B
C	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	-0.006	+0.000	+0.000	+0.000	+0.000	C
D	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	-0.005	+0.000	+0.000	+0.000	+0.000	+0.000	D
E	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	<u>+0.018</u>	+0.000	+0.000	+0.000	E
F	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	F
G	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	G
H	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	+0.000	H
	1	2	3	4	5	6	7	8	9	10	11	12	

overnight & very strong SE9

NN

(Mice) NOS3 potential positives

12/23 ELISA

12/24 Microscope

2B6

+

2F3

+

4FY

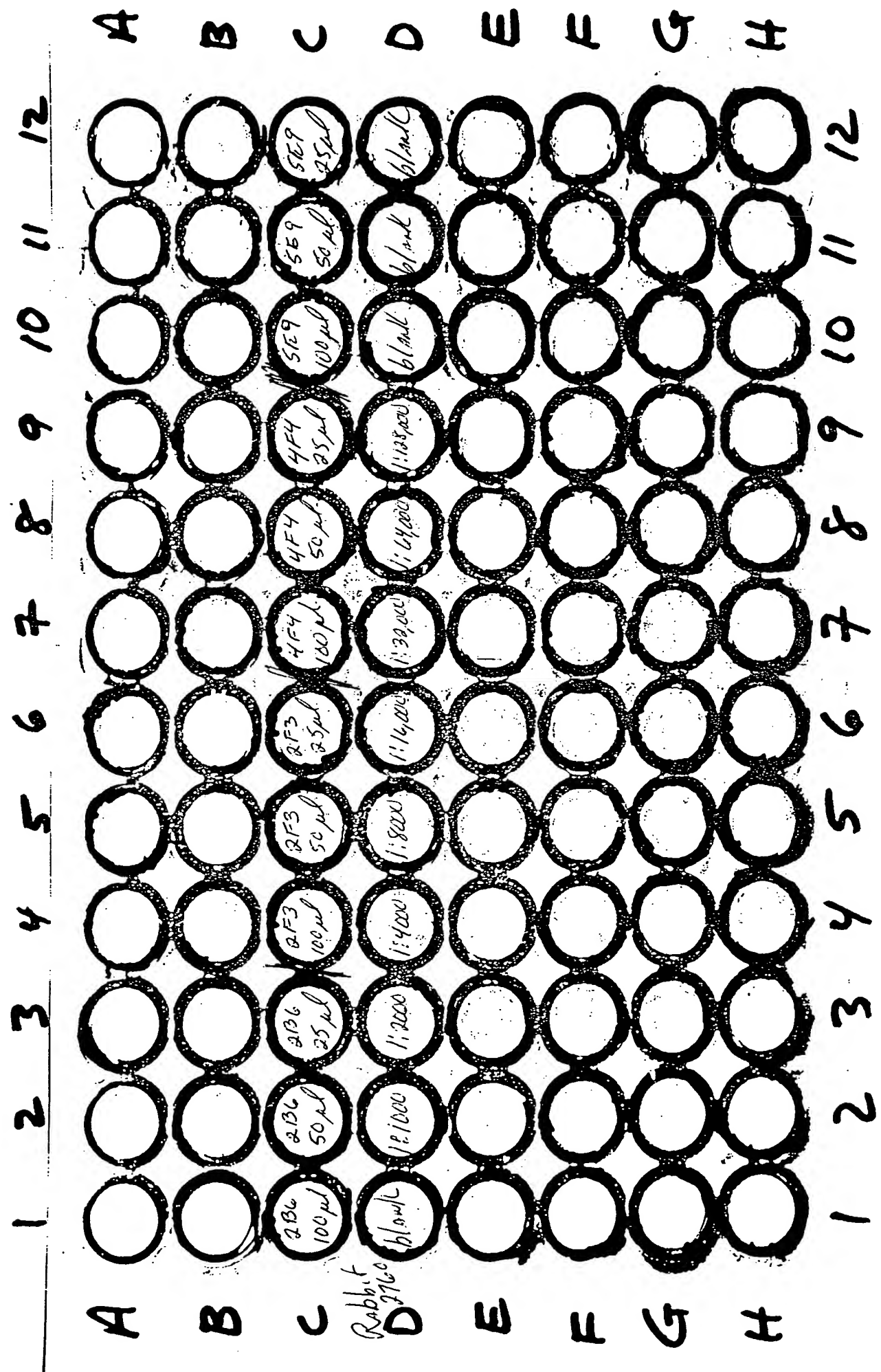
+

5E9

+

Testing Positive Hybrids
 VS. ADP Sephadex pure h.wos
 @ 10 ng/well

12/27/93



Rabbit
 2760

EASY BEAM

Plate # rhINOS Date 12/28/93 Filter 492 nm
 Operator DF Comment ADP Sepharose pure human iNOS
@ 10 ng/well

	1	2	3	4	5	6	7	8	9	10	11	12	
A	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	A
B	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	B
C	100µl 286	50µl	25µl	100µl 2F3	50µl	25µl	100µl 4F4	50µl	25µl	100µl 6E9	50µl	25µl	C
	+0.005	+0.000	+0.010	+0.000	+0.006	+0.040	+0.014	+0.016	+0.023	+0.015	+0.013	+0.009	
D	Blank 1:1000	1:2000	1:4000	1:8000	1:16000	1:32000	1:64K	1:128K	1:256K	1:512K	1:1024K	1:2048K	D
	+0.006	+1.684	+1.574	+1.311	+1.032	+0.537	+0.272	+0.112	+0.020	+0.000	+0.000	+0.000	
E	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	E
F	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	F
G	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	G
H	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	=====	H
	1	2	3	4	5	6	7	8	9	10	11	12	

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